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# Salt tolerance in mungbean is influenced by application of phosphorus in sand culture

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**Abstract**
Salinity stress is major abiotic stress which limits growth and productivity of plants in many areas of the world. Nutrient imbalance on the other hand, is one of the major abiotic constraints limiting productivity of pulses. Mungbean is ecologically important legume crop and its susceptibility towards salinity stress has limited the production. Fertilization management is an important technique to alleviate the adverse effects of salinity stress on plants. The present study was carried out to assess various effects of phosphorus application on salt tolerance and to determine effect of phosphorus on morphological and biochemical parameters of mungbean under induced salt stress. Mungbean genotypes were sown in plastic pots filled with sand and grown with recommended packages of practices. It was found that phosphorus (2mM) alleviated salt stress (50mM) as morphological and biochemical parameters showed increased responses when phosphorus was applied along with salt stress. Thus, it can be concluded that phosphorus mitigates the effects of induced salt stress responses in mungbean.

**Keywords:** Mungbean, phosphorus, salinity, salt tolerance, sand culture

## Introduction
Different environmental stresses viz., high winds, extreme temperatures, soil salinity, drought and flood have affected the productivity and cultivation of agricultural crops but among these salinity stress is the most catastrophic environmental stresses, which causes major reductions in cultivated land area, crop productivity and quality (Yamaguchi and Blumwald, 2005, Shahbaz and Ashraf, 2013) [21,19]. It is one of the major abiotic stresses, especially in arid and semi-arid regions and can severely limit plant growth and yield (Parvaiz and Satyawati, 2008) [16]. Salt stress causes adverse effects on the physiology of plants, leads to death of plant resulting in growth arrest and metabolic damage, osmotic effects, ion toxicity and nutrient imbalance. Phosphorus is a key element involved in various functions in growth and metabolism of pulses. Phosphorus uptake and concentration decreases in salinity conditions exhibiting symptoms like reduced and stunted growth, dark green coloration of the leaves, production of slender stems, and death of older leaves in plants (Taiz and Zeiger, 2006) [20]. NaCl reduces activity of P in the soil by inducing high ionic strength. Pulses are more sensitive to salinity than cereals and oilseed crops. Mungbean is an important short duration legume crop of high nutritive values and nitrogen fixing ability. It belongs to genus *Vigna.* Abiotic stress severely reduces growth and development of all pulses including mungbean. Mungbean is sensitive to salinity stress and it drastically affects morpho-physiological and biochemistry of mungbean crop. General stunting, chlorosis and necrosis are commonly reported symptoms of salt stress. Besides, it also reduces chlorophyll content, protein and nutrient availability. One of the important techniques in alleviating the adverse effects of salt stress on plants is proper fertilization management practices (Colla *et al*. 2008) [7]. On this note, the present study is basically focused on the effect of salinity stress on morphological and biochemical attributes of mungbean and the influence of phosphorus on the same, in sand culture.

## Materials and Methods
### Sand sterilization and pot experiment
Sand was collected from construction site, Institute of Agricultural Sciences, Banaras Hindu University, and sieved, washed with hot water and sterilized with formaline solution in 1:50 ratio and washed thoroughly. Sand was filled in pots and pot experiment was conducted in net house condition with suitable precautions.

### Plant material, Growth conditions and Stress and phosphorus treatments
Seeds of mungbean (*Vigna radiata* L. Wilczek) variety HUM-16 were procured from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi. These were surface sterilized with 0.01% $HgCl_2$ for 3-5 min and washed

extensively and sown in plastic pots containing sterilized sand. Seeds were allowed to germinate and pots were watered at 1-2 days interval depending upon moisture status of the sand. Phosphorus solution of 0.75mM and 2mM were prepared. 0.75mM P was applied at leafy stage of plant in treatments $T_2$, $T_6$ and $T_8$ while 2mM P was applied in treatments $T_3$, $T_7$, and $T_9$, respectively. Similarly solution of 50mM and 100mM NaCl was prepared and salt stress conditions were created by applying 50mm NaCl to treatments $T_4$, $T_6$, $T_7$, and 100mM to treatments $T_5$, $T_8$, $T_9$, respectively 30 days after sowing when plants became hardy. The Hoagland solution was given as a nutrient solution to the pots. In addition, seedlings were washed and used for several morphological and biochemical analysis before and after ten days of stress imposition.

**Measurement morphological parameters**

About 3 seedlings were selected from each treatment before and after ten days of stress treatment and washed thoroughly with water. Shoot length was measured with the help of centimeter scale in ten different treatments with three replications. Leaf area of ten independent treatments with replications was recorded using Leaf Area Meter. Leaves of each treatment and replications were washed thoroughly, blotted gently and weighed under weighing balance in order to obtain fresh weight. For dry weight, leaf sample and shoot samples were dried in oven at 100˚C for an hour and then at 70˚C for 72 h and finally dry weight of seedlings was noted.

**Treatment details**

| Treatment Number | Treatment details |
|---|---|
| T | Control(Hoagland solution without $KH_2PO_4$) |
| $T_1$ | Control(Normal Hoagland solution) |
| $T_2$ | 0.75mM P + H.S without $KH_2PO_4$ |
| $T_3$ | 2mM P + H.S without $KH_2PO_4$ |
| $T_4$ | 50mM NaCl + Normal H.S |
| $T_5$ | 100mM NaCl + Normal H.S |
| $T_6$ | 0.75mM P+ 50mM NaCl + H.S without $KH_2PO_4$ |
| $T_7$ | 2mM P + 50mM NaCl + H.S without $KH_2PO_4$ |
| $T_8$ | 0.75mM P+ 100mM NaCl + H.S without $KH_2PO_4$ |
| $T_9$ | 2mM P+ 100mM N 2mM P + 100mM NaCl + H.S without $KH_2PO_4$ |

Where, H.S. stands for Hoagland solution

**Estimation of chlorophyll content**

Chlorophyll content was determined in leaf sample by DMSO method (Hiscox and Israelstam, 1979). 50 mg leaf sample was put in 50ml DMSO and kept in Oven at 72˚C for 2 h which after cooling down was made to 10 ml with DMSO and absorbance was taken at 663 and 645 nm using spectrophotometer.

**Estimation of proline content**

The proline content was estimated by Bates *et al* (1973).

**Estimation of protein content**

Protein content was determined by Coomassive Brilliant Blue G-250 dye binding method.

**Estimation of sugar content**

Sugar content in leaves before and after imposition of salt was determined by anthrone reagent method (Sadasivam and Manickam, 1992).

**Estimation of phosphorus content**

Phosphorus in an aliquot was determined using methods based on molybdophosphoric blue color developed by reduction of heteropoly complex or by vandomoly bdophosphoric yellow color method.

***Di-acid digestion***

0.1 gm leaf sample was weighed in flask and to it 10 ml of conc. $HNO_3$ and 2-3 ml $HClO_4$ was added. The set up was kept on hot plate in acid-proof digestion chamber and sample was digested until it became colour less and only white dense fumes appeared. The flask from digestion chamber was removed and allowed to cool, after that 30 ml distilled water was added and filtered through What man No.42 filter paper, the aliquot transferred into a 100 ml volumetric flask.5 ml of aliquot was transferred into 50 ml volumetric flask and added 5 ml vandate- molybdate reagent and diluted to 50 ml with distilled water, and absorbance was recorded at 420 nm with the help of spectrophotometer.

**Results and Discussion**

**Effect on morphological and biochemical parameters**

It was observed that shoot length significantly decreased with increase in salt concentration compared as shown in $T_4$ and $T_5$. In presence of NaCl, shoot length was recorded highest in treatment $T_7$ at phosphorus concentration of 2 mM. Abu-Rom man *et al*. (2013) [1] reported similar trend of results in cucumber micro shoots. It may probably be due to nutritional role of phosphorus, and treatment with the same, decreased the negative effects of salinity in terms of growth indices. Salinity, on other hand, decreased length of the shoot as reported in treatment $T_4$ and $T_5$.Leaf area characterizes crop canopy. Salinity resulted in decrease in leaf area as shown in treatment $T_4$ and $T_5$ which was increased with application of phosphorus. Alam *et al*. (2010) [2] reported that leaf area increased with the integration of the organic and inorganic sources (FYM, poultry manure and chemical P fertilizer) in mungbean. Ali *et al*. (2004) [3] also reported decrease in leaf area in rice genotypes in response to salinity. Fresh weight and dry weight of leaves were affected severely under saline conditions as in treatments $T_4$ and $T_5$, and increased in treatment $T_7$ over other treatments in presence of NaCl. Sima *et al*. (2012) [11] reported decreased dry weight of shoots in barley species. Chlorophyll a and b content followed similar pattern of results; their content increased significantly by interaction of phosphorus with NaCl in treatment $T_7$ (NaCl 50mM + 2mM P) as compared to other treatments such as $T_6$, $T_8$, $T_9$. The lowest chlorophyll content was observed in $T_5$ (100mM NaCl) followed by $T_4$ (50mM NaCl) indicating that salinity decreases the chlorophyll content. The loss of

chlorophyll under salt stress could be related to photo inhibition or ROS formation (Kato and Shimizu, 1985) [9]. Parida and Das (2005) [15] suggested the decrease in chlorophyll and carotenoid content of leaves in response to salt stress as a general phenomenon. Under salinity stress plants accumulate compatible solutes such as proline for their osmo protection activity (Chelli-Chaabouni *et al*. 2010) [6]. The result of present study showed that increase in salt concentration led to increased proline content. Similar results were shown by Rahneshan *et al*. (2018) [18] in pistachio *(Pistacia vera* L.). The decrease in protein content is due to the effects of sodium chloride on protein synthesis. (Omar *et al*., 1993) [14]. There is a significant increase in protein content in leaves of $T_7$ (NaCl 50mM + 2mM P) over all other treatments in presence of NaCl while the treatments $T_4$ and $T_5$ showed significant decrease in protein content where NaCl was applied. The decrease in protein content with highest concentration of salinity was reported by Astorga *et al*. (2010) [4]. There was a significant increase in sugar content in leaves in $T_3$ in presence of phosphorus concentration 2mM and reported least in saline imposed treatments. The concentration of phosphorus decreases during salinity stress, with maximum decrease in P observed in case of $T_5$ followed by $T_4$, as salinity stress decreases the uptake and concentration of P in plant tissues by increasing the concentration of $Na^+$ and $Cl^-$. Abu-Romman *et al*. (2013) [1] stated that NaCl-induced salinity decreased P uptake and increase in P concentration resulted in a significant increase in P uptake.

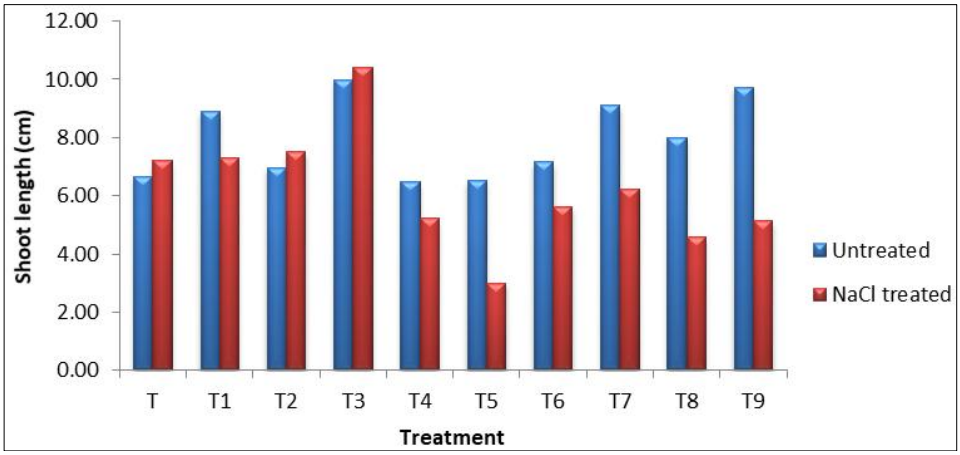


**Fig 1:** Effect of phosphorus on shoot length (cm) in *Vigna radiata* (L.) under induced salt stress

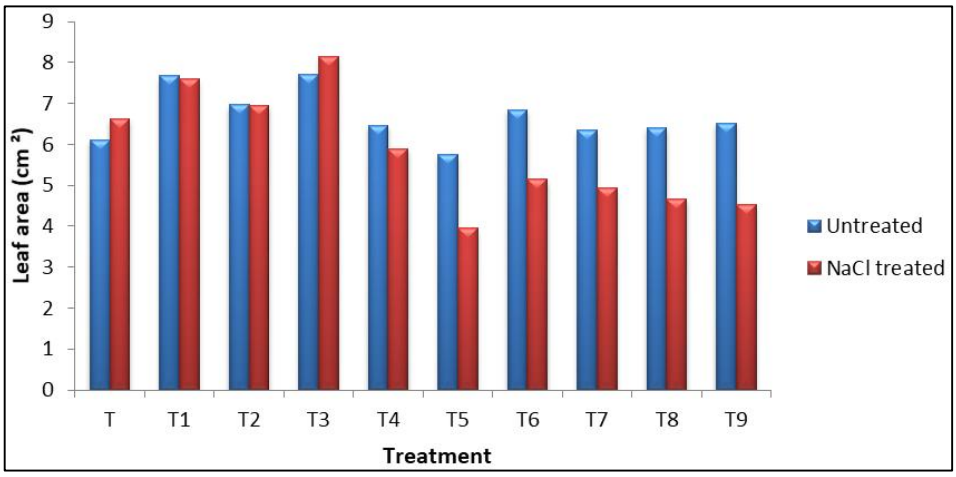


**Fig 2:** Effect of phosphorus on leaf area ($cm^2$) in *Vigna radiata* (L.) under induced salinity stress

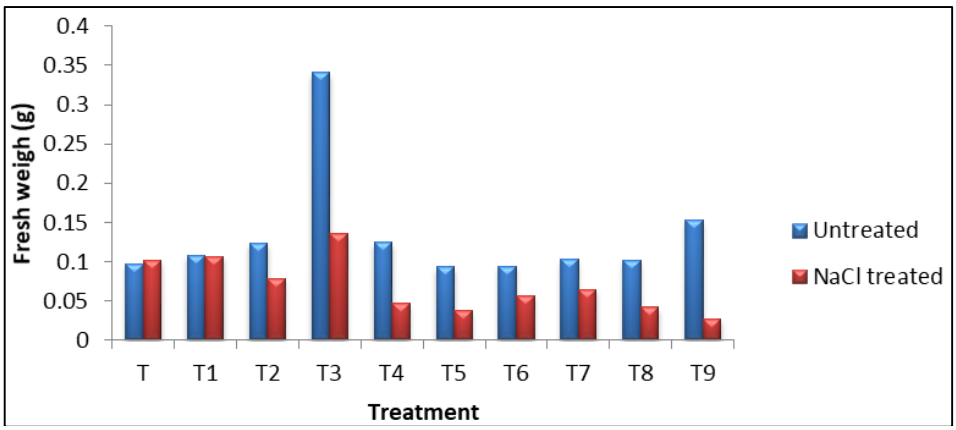


**Fig 3:** Effect of phosphorus on fresh weight (mg $g^{-1}$ FW) in *Vigna radiata* (L.) under induced salinity stress

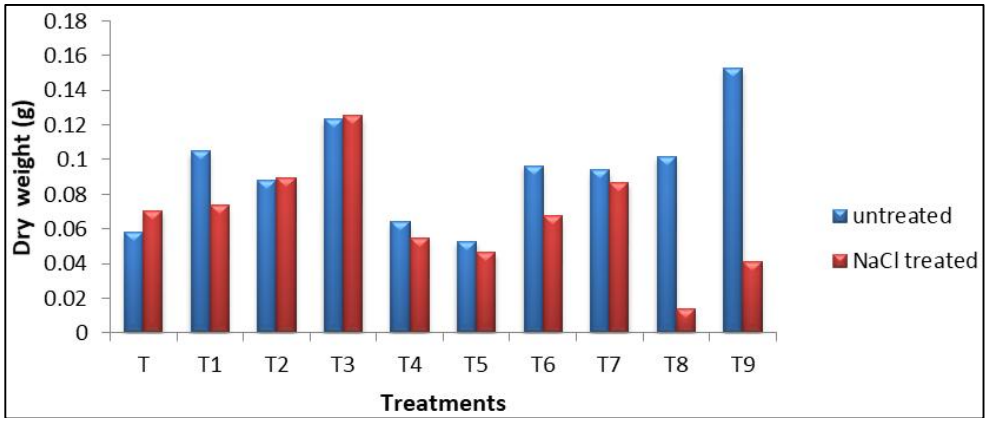


**Fig 4:** Effect of phosphorus on dry weight of shoots (g) in *Vigna radiata* (L.) under induced salinity stress

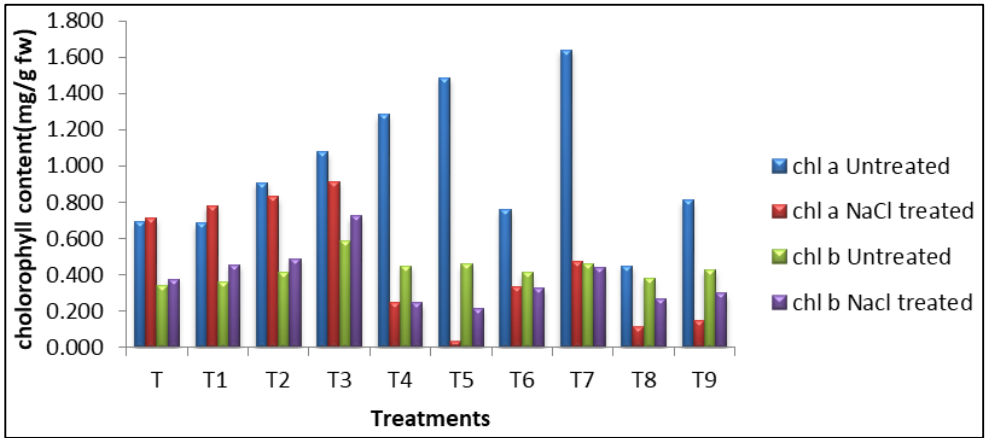


**Fig 5:** Effect of phosphorus on chlorophyll a and b content (mg $g^{-1}$ FW) in *Vigna radiata* (L.) under induced salinity stress

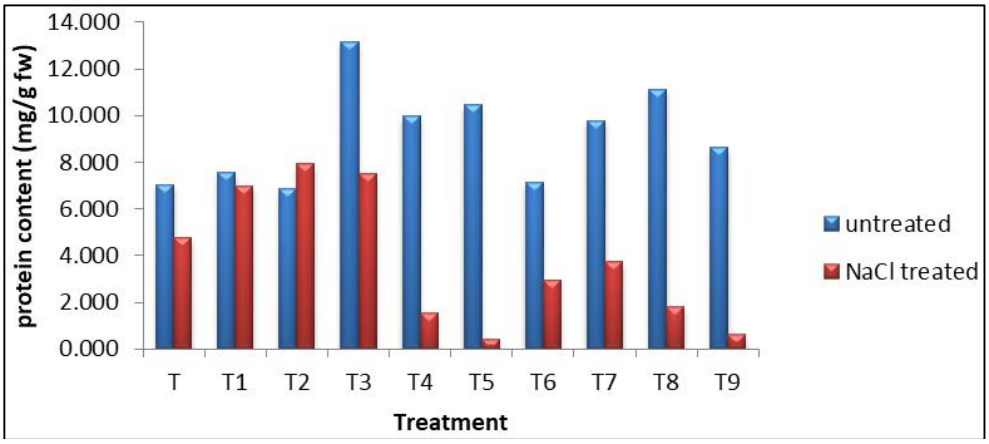


**Fig 6:** Effect of phosphorus on protein content (mg $g^{-1}$fresh weight) in *Vigna radiata* (L.) under induced salinity stress

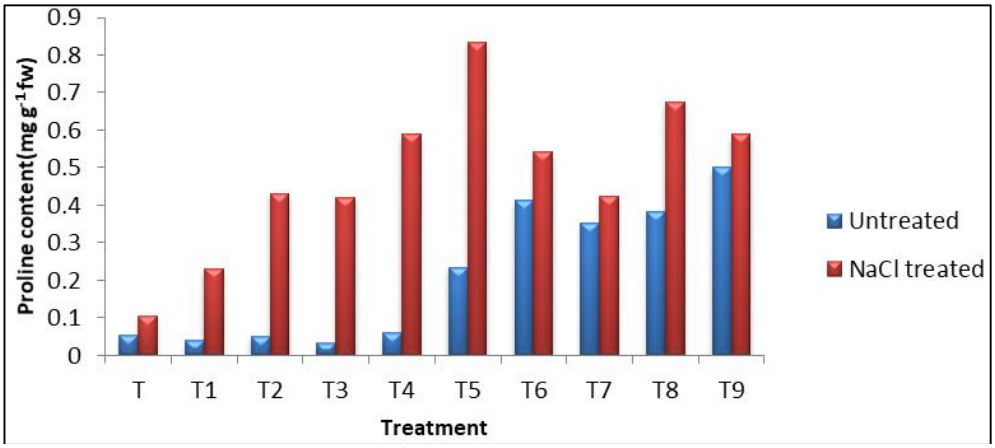


**Fig 7:** Effect of phosphorus on proline content (mg $g^{-1}$ FW) in *Vigna radiata* (L.) under induced salinity stress

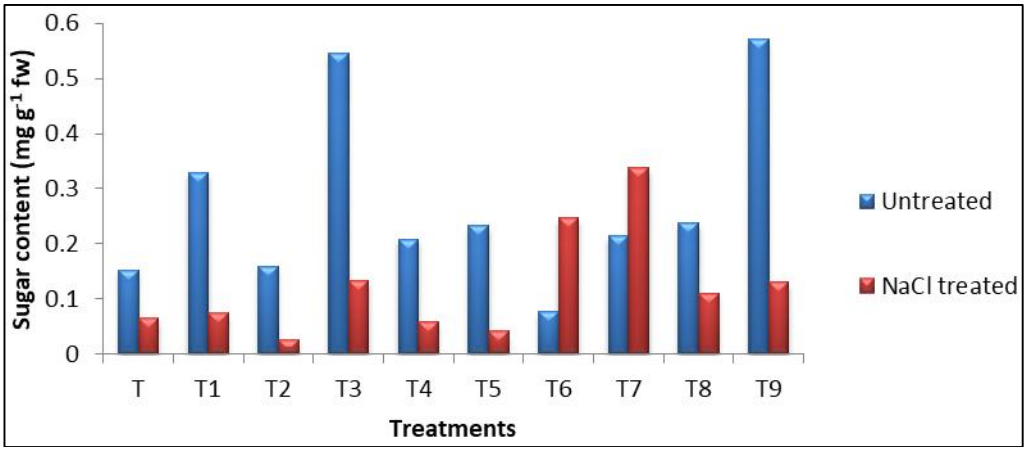


**Fig 8:** Effect of phosphorus on sugar content (mg $g^{-1}$ FW) in *Vigna radiata* (L.) under induced salinity stress

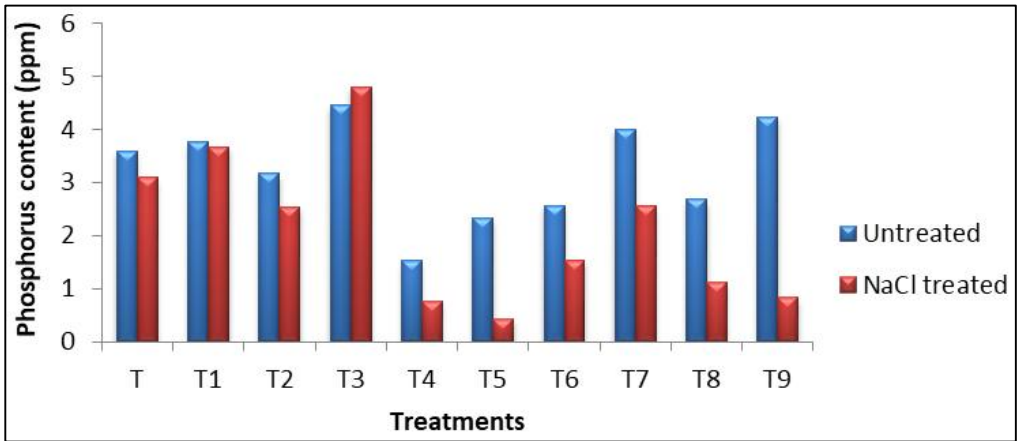


**Fig 9:** Effect of phosphorus content (ppm) in *Vigna radiata* (L.) under induced salinity stress

## Conclusion

It is concluded that when plants are subjected to salinity stress, nutritional disorders may develop. If nutrient deficiency is the severe growth-limiting factor, then growth may be improved by addition of proper nutrients. Phosphorus alleviates the salt stress. Studies show that there are positive effects between soil salinity and amount of phosphorus consumption in improving plant function (Bates and Lynch 2000) [5], Naheed *et al*., (2008) [13]. The interaction between salinity and P affected plant growth, and by increasing P, there was an increased salt tolerance Kaya *et al*. (2001) [10] also reported that application of high P ameliorated damage of salinity stress in cucumber and pepper. Phang *et al*. (2009) [17] and Zribi *et al*. (2011) [22] also reported that high external P increased Na uptake and reduced salt tolerance of soybean and barley. In the present study, phosphorus application at the concentration of 2 mM was most effective in combating salinity caused up to 50 mM (NaCl). Improved salt tolerance through P fertilization is likely a promising strategy to improve plant salinity tolerance and thus productivity. Phosphorus has profound effect on salinity stress. There is a need to conduct further elaborate study with wide range of salt and phosphorus concentrations in the field conditions to better demonstrate the interaction of P and salt on the plants.

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